

Coastal Benthic Optical Properties (CoBOP): Characteristics and Processes Related to Optical Properties of Benthic Marine Organisms and Substrates

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LONG-TERM GOALS

The long-term goal of this research is to gain an understanding of the nature and significance of fluorescence and reflectance characteristics of benthic marine organisms in general, and coral reef cnidarians in particular. We wish to determine both how biological processes act to determine the optical properties and how optical measurements can be used to provide insight into biological state or process.

OBJECTIVES

The objectives for this year's work were to:

- Refine the measurement and mathematical techniques for separating the fluorescence and reflectance components contributing to spectral signatures under daylight illumination;
- Seek spectral evidence for the relationships among or functions of coral fluorescent pigments
- Investigate the within-colony and within-species variability of fluorescence in corals.

APPROACH

This work is part of the Coastal Benthic Optical Properties (CoBOP) program. The main effort in FY99 was conducted as part of the CoBOP field campaign at the Caribbean Marine Research Center, Lee Stocking Island, Bahamas. The work described here was carried out by Eran Fux (doctoral candidate, Department of Ocean Engineering, MIT) under the supervision of the Principal Investigator.

In situ measurements of fluorescence and reflectance were made with the Benthic SpectroFluorometer (BSF) (Mazel, 1997a), a diver-operated instrument for measurement of spectral signatures from discrete benthic features. Laboratory measurements of fluorescence excitation and emission spectra were made with a FluoroMax-2 spectrofluorometer.

From the *in situ* measurements we identified a number of specimens that contained only one of the coral host fluorescent pigments (Mazel, 1997b). These specimens were brought to the laboratory for measurement of excitation and emission spectra, apparent fluorescence yield, and 'true reflectance' (separating the fluorescence contribution from the reflectance contribution). We also collected specimens that contained the blue-green- and green-fluorescent pigments and made fluorescence emission measurements at a series of excitation wavelengths to investigate the apparent coupling

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between these two pigments (Fux and Mazel, 1999). Measurements were made from other specimens to investigate hypotheses about the possible function of the coral fluorescent pigments.

A series of *in situ* fluorescence emission measurements were made from the top and sides of colonies of the coral *Montastraea cavernosa* to explore the question of within- and between-colony variability in fluorescence characteristics.

WORK COMPLETED

We participated in the CoBOP field campaigns in January and May/June 1999. *In situ* and laboratory measurements were made to address the stated objectives. Data analysis is well advanced. Preliminary results of part of this work (Fux and Mazel, 1998) was presented at the Ocean Optics XIV conference in November, 1998.

RESULTS

The techniques for separating the reflectance and fluorescence components of the light leaving the coral surface have been better refined. Combining this information with our measurements of fluorescence efficiency we are able to predict the spectrum of light leaving the surface of a fluorescing coral (or other specimen) under different illumination conditions. Figure 1 is an example of the need to take the two components into account. Starting with lab-measured reflectance data, the spectral signature at 8 m depth was predicted in two ways: 1) simply applying the new illumination spectrum to the apparent reflectance, without separation of components (dashed line), and 2) computing the contributions of the fluorescence and reflectance components separately (thin solid line). The spectrum predicted by the second approach is in much better agreement with the spectrum measured *in situ* (thick solid line).

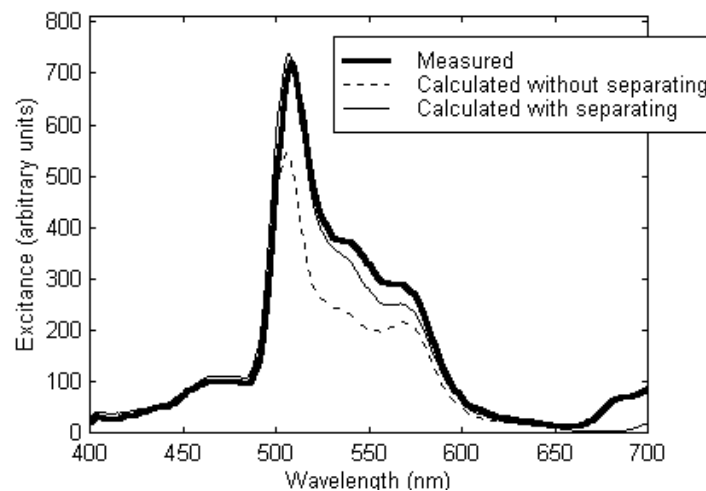


Figure 1. Prediction of coral spectral signature at 8 m depth with (thin solid line) and without (dashed line) separating the fluorescence and reflectance components. Measured data is shown as a thick solid line.

We made 103 *in situ* fluorescence emission scans from 19 colonies of the coral *Montastraea cavernosa*. Measurements were made from tops and sides of the colonies, recording depth and

orientation. No consistent fluorescence pattern was found. Some colonies contained only one of the common coral fluorescent pigments (in addition to chlorophyll in the symbiotic algae), while others contained various combinations of pigments. No correlation with orientation or depth could be found. The reason for and possible significance of this variability is not known.

Our prior measurements of the contribution of fluorescence to reflectance suggested that the coral fluorescent pigments might be masking the symbiotic algae, removing photons that might otherwise be used for photosynthesis. This would contradict suggestions that have been made that the function of the fluorescence is to aid photosynthesis by converting more photons to wavelengths that can be absorbed by chlorophyll or photosynthetic accessory pigments. We measured excitation spectra for chlorophyll fluorescence in fluorescent and non-fluorescent specimens to explore this. For the green fluorescence (emission peak approximately 515 nm, (Mazel, 1997b)) we did not find any examples in which the excitation of that pigment contributed an ‘extra boost’ to the chlorophyll fluorescence, as would be expected if energy were being transferred. In contrast, we found evidence (Figure 2) in one strongly fluorescent specimen that the green pigment does appear to be blocking energy that might otherwise be used for photosynthesis.

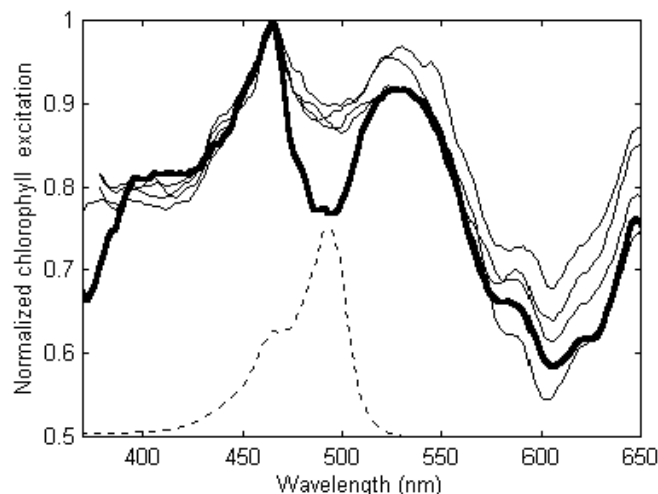


Figure 2. Excitation spectra for chlorophyll fluorescence in corals. The spectra represented by the thin solid lines were measured from corals with no strong fluorescent pigments in the coral tissues, while the thick solid line was measured from a strongly green-fluorescent specimen. The excitation spectrum for the green fluorescence (dashed line) is included to show the correlation between the absorption by the green-fluorescent pigment and the dip in the chlorophyll excitation spectrum.

Through our spectral measurements we have also demonstrated significant energy transfer from the blue-green-fluorescent (emission peak approximately 486 nm (Mazel, 1997b)) to the green-fluorescent pigment. We were able to determine a constant factor for any given specimen (although highly variable between specimens) that related the number of photons fluoresced by the blue-green pigment to the number of photons fluoresced by the green pigment that originated as photons absorbed by the blue-green pigment. Further analysis enables us to estimate the coupling efficiency for this transfer, defined as the fraction of photons absorbed by the blue-green pigment that produce excitation of the green pigment. The high values of this efficiency (as high as 25%) indicate that the energy transfer is not a trivial emission-reabsorption process, but rather an energy transfer at a more meaningful level.

This suggests a biochemical relationship between these pigments, but the function of that relationship is not known.

IMPACT/APPLICATION

The work described here further elucidates the nature and possible function of fluorescence in corals and other marine organisms. The separation of the true reflectance and fluorescence contributions to spectral signatures, coupled with measurements of fluorescence yield, provide the data needed to predict how those signatures will vary as a function of ambient illumination. The results are directly relevant to interpretation of ambient-illumination measurements of spectral signatures and irradiance reflectance that are being made by collaborating CoBOP researchers.

The spectral measurements that address the possible function of the coral fluorescent pigments may contribute to reaching an understanding of the nature of these pigments and the factors that control their expression. This would have implications for ecological monitoring and assessment.

TRANSITIONS

We are working with CoBOP researchers Dave Costello and Robert Maffione to model the responses of their instruments under ambient illumination conditions. The results concerning fluorescent pigment function and energy transfer are being used in work we are conducting with Paul Falkowski and Michael Lesser to identify these pigments and their role in coral physiology.

RELATED PROJECTS

We are investigating other aspects of benthic optics through a related CoBOP project. We are also developing new instrumentation for making the *in situ* optical measurements.

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PUBLICATIONS

Fux, E., and C. H. Mazel. 1999. Unmixing coral fluorescence emission spectra and predicting new spectra under different excitation conditions, *Applied Optics*, 38:486-494.